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Final Technical Report

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Candidate Species Selection for a Controlled Ecological Life-Support System

research conducted by

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Technical Monitor

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NASA Ames Cooperative Agreement NCC 2-100 was a project funded in the biomass production group of the NASA Controlled Ecological Life-Support System (CELSS) program. The early years of the project emphasized the use of leaf lettuce (*Lactuca sativa* L.) as a model crop system to optimize photosynthetic productivity and yield.

There were a number of reasons for choosing leaf lettuce as a model crop for biomass productivity studies. For one thing, it is a leafy salad vegetable, and as a strictly vegetative crop its photosynthetic productivity is not complicated by source/sink relationships and monocarpic senescence as are crops that depend upon reproductive development. It not only has a high harvest index (ratio of edible to total biomass) $\geq$ 80%, leaf lettuce has a short production schedule (28 to 35 days under non-optimized controlled environments). Lettuce is readily adaptable to soilless, hydroponic culture, and is one of the few crop species that is tolerant of NH_4^+ in nutrient solutions. From a nutritional perspective, lettuce provides a few vitamins, minerals, and some fiber for the diet, but its main value in a CELSS would be to provide variety, freshness, and texture in a mainly vegetarian diet. Lettuce was selected early in the project when it was felt that a vegetarian diet consisting of either a generous or at least a modest selection of species would be available to support human crews in a CELSS. It was, therefore, selected mainly as a model crop to explore maximization of photosynthetic productivity and to psychologically augment the diet of human crews in space.

Initial studies involved screening a variety of cultivars under controlled-environment conditions in growth chambers. Environmental conditions that were varied included photosynthetic photon flux, photoperiod, day/night temperature, and hydroponic culture conditions including strength of nutrient solution and form and concentration of nitrogen in nutrient solution.

Conditions found to generally favor rapid growth rate included high photosynthetic photon flux (PPF) up to 900 $\mu mol\ m^{-2}\ s^{-1}$ (400 to 7000 nm), long photoperiods up to 20 h day^{-1}, continuous 25°C air temperatures, and single-strength Hoagland's nutrient solution containing double-strength nitrogen as 5 mM NH_4^+ + 25 mM NO_3^-. Using optimizing environments such as these, cultivars such as 'Waldmann's Green', 'Salad Bowl', and 'Black-Seeded Simpson' responded positively in terms of growth and yield, whereas cultivars like 'Grand Rapids' and 'Ruby' were relatively non-responsive. The most critical conditions leading to growth stimulation were the combination of high PPF from very high output fluorescent lamps (82% of input wattage) plus frosted incandescent bulbs (18%) together with elevated N in nutrient solution as NH_4^+ + NO_3^-. This combination had a synergistic effect on growth of responsive cultivars. These initial experiments were conducted in two reach-in Sherer-Gillette growth chambers.

Because lettuce is a leafy vegetable crop, it was felt that conditions which promote photosynthesis should enhance growth rate and yield. It therefore became important to be able to measure photosynthetic gas exchange without having to remove plants from the modified controlled environments in which they are growing. It also became apparent that it was necessary to measure gas exchange of crop canopies rather than of individual leaves or individual plants, as is done more customarily. This need led to the creation and evolution of the "Minitron" system, which is a combination controlled-environment plant-growth chamber/small canopy cuvette for measuring gas exchange under undisturbed growth environments. The Minitron chambers developed for CELSS crops are transparent vertical cylinders 24 inches in diameter and 24 inches high. They have internal temperature control and air circulation capability, as well as a hydroponics system that permits compartmentalization and separate measurement of root-zone and shoot-zone gas-exchange rates. Carbon dioxide enrichment of the shoot atmosphere is achieved by injecting measured amounts of CO_2 into the airstream that is humidified and injected into the chamber. Flow rate and CO_2 injection are controlled by a series of mass-flow control values under computer control. A full proportional-integral-derivative (PID) program maintains CO_2 in the atmosphere at a constant level in spite of a slow turnover rate of atmosphere through the chamber and a variable rate of removal by growing crop canopies. Photosynthetic rate is determined by the amount of CO_2 injected to maintain constant CO_2 in the outflow atmospheres that goes through an infrared gas analyzer, which in turn also communicates with the computer forming a complete CO_2 control-gas-exchange-measuring control loop. Transpiration rates are measured with in-line dew-point humidity sensors using the computer as a data-logging/number-crunching device. The Minitron System was used as a semi-open gas-exchange system.

After developing the Minitron System and getting it up to full capability, 'Waldmann's Green' leaf lettuce canopies were grown under a variety of controlled environments, particularly featuring atmospheric CO_2 enrichment, use of different plant-growth lamps, and growth-regulating chemicals. The general findings of these studies were that hydroponically grown lettuce does not respond significantly to elevated PPF or to CO_2 enrichment for the first 11-12 days of seedling development following seed germination, which corresponds to the lag phase of a sigmoid growth curve. Thus, optimizing conditions could be conserved during the lag phase. However, as the plants moved into rapid exponential growth (days 12-17) they responded dramatically and rapidly to high PPF from the right type of lamps and to the right level of CO_2 enrichment. Periodic, destructive sampling of whole plants at different stages of the growth curve permitted measurements of leaf area and dry weights at different stages of development, permitting calculation of the growth-dynamics parameters relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR), as well as instantaneous photosynthetic rates (Pn). The hydroponics lid of the Minitron was designed such that this sampling amounted to a uniform thinning of the leaf canopy such that it would quickly close again due to normal growth and development.

The optimum level of CO_2 was highly interactive with PPF. All levels of enrichment tested up to 2000 ppm enhanced RGR and Pn, but maximum stimulation occurred at 1500 ppm when used together with high PPF. Concentrations above 2000 ppm strongly inhibited RGR, NAR, and Pn. Leaf lettuce was very particular about the type of plant-growth lamp it would respond to. It was fairly unresponsive to elevated PPF from fluorescent alone or to metal halide alone, but when 84% of the irradiance was provided by incandescent lamps, lettuce grew at extremely high rates in the presence of high CO_2 and N enhancement in the nutrient solution. In fact, RGRs > 1 (i.e., more than doubling dry mass in a single day) were recorded during the brief period of exponential growth. From day 17, RGR declines abruptly, regardless of environment, indicating that it is a waste of power to maintain high PPF beyond the brief period of exponential growth. The plants become increasingly unresponsive to optimizing environments beyond the inflection point in the sigmoidal growth curve. Since CO_2 is much cheaper than power for lights, some yield and cost benefits may be realized by extending CO_2 enrichment at least into the start of the plateau phase, but PPF can be cut back to at least 400 μmol m^{-2} s^{-1}.

The spectral component of incandescent radiation that stimulates leaf lettuce growth so significantly under the aforementioned conditions is unknown, but is likely to be the red and/or far-red wavebands. From a photosynthetic standpoint, red is a strong candidate, from a leaf-expansion standpoint, red and near far-red are candidates. Incandescent radiation contains virtually no blue emissions. Once the key spectral component is known, development of a radiation source with much improved luminous efficacy in the appropriate spectral region could make the rapid controlled environment production of leaf lettuce energetically feasible. This approach could be extremely valuable with other CELSS candidate species as well.

In related studies conducted in a walk-in growth chamber with 'Black-Seeded Simpson', a pale-green cultivar of leaf lettuce, high PPF in the range of 1000 $\mu mol\ m^{-2}\ s^{-1}$ of photosynthetically active radiation (PAR) provided by a combination of (orange-biased) high-pressure sodium (HPS), (blue-biased) metal halide (MH), and (incandescent) quartz iodide (QI) lamps stunted and yellowed the growth of the plants growing in a recirculating hydroponics system. When grown under 400 $\mu mol\ m^{-2}\ s^{-1}$ from the MH + QI alone the plants were normal color and grew at an acceptable rate. When as much as 12 hours of an additional 660 $\mu mol\ m^{-2}\ s^{-1}$ of PAR from HPS lamps was superimposed on the MH + QI radiation, no additional growth took place but the plants became yellower. If the dosage of HPS was extended beyond 12 h day^{-1} and up to 20 h day^{-1}, growth actually was greatly stunted and became increasingly chlorotic. When HPS was used as the sole source of plant-growth radiation at several different PPFs, the results indicated that PPFs of 600 $\mu mol\ m^{-2}\ s^{-1}$ and above actually were detrimental to lettuce growth, and below that level permitted a certain baseline of growth to occur until at low levels it became growth limiting. Although HPS is a long-lived high-intensity discharge (HID) lamp type with good output stability, it clearly is not a good choice of lamp with which to attempt to grow lettuce under optimizing conditions.

Several approaches were used in effort to shorten the lag phase of the sigmoid growth curve of lettuce. The idea is that if the onset of exponential growth can be hastened by even 1 or 2 days, it could stimulate a 50 to 100% increase in edible biomass by the end of the typical 19 to 23-day production cycle. The plant-growth regulator triacontanol applied as a spray of 10^{-7} M active ingredient to 4-day-old seedlings enhanced leaf dry weight as much as 20% six days after application. Combined with optimizing conditions of CO_2 enriched to 1500 ppm and PPF enhanced to 900 μmol m^{-2} s^{-1}, crop growth rates (CGRs) from 10 to 19 days were enhanced 5 to 10%. Triacontanol as a stable colloidal dispersion has potential for mildly stimulating the yield of hydroponically grown leaf lettuce under optimizing controlled-environment conditions. In another study, gibberellic acid (GA_3) at 1 μM brushed on young, expanding leaves of lettuce stimulated shoot dry weight, leaf area, and specific chlorophyll content of seedlings in lag phase without causing stem bolting. Application of low concentrations of GA_3 directly to leaves holds promise for hastening the onset of exponential growth and increasing plant size at the standard harvest data (or hastening harvest).

The best combinations of optimizing conditions for lettuce have resulted in crop growth rates of more than 60 g DW edible biomass m^{-2} growing area day^{-1} during exponential growth. Our best experiment gave a CGR of 67 g m^{-2} day^{-1} for edible biomass. CGR for total biomass in that experiment was 80.4 g

m^{-2} day^{-1}. Cultural and environmental conditions required to reach at least 60 g m^{-2} day^{-1} for leaf lettuce include the following:

- 13.5-cm spacing of plants
- continuous 25°C air temperature
- 85% relative humidity
- 20-hour photoperiod
- 900 μmol m^{-2} s^{-1} of PAR from 84% incandescent + 16% fluorescent radiation from days 11-19
- 1500 ppm CO_2 from days 11-19
- Hoagland's nutrient solution, pH 6.0 $\pm$ 0.2:
  - half-strength with 7.5 mM N as NO_3^- alone from days 0 to 5
  - single-strength with 15 mM N as NO_3^- alone from days 5 to 10
  - single-strength with 30 mM N as 5 mM NH_4^+ + 25 mM NO_3^- from days 11-19

During the latter part of the project period of NCC 2-100, the CELSS Program Manager (James Bredt) suggested that CELSS vegetarian diets might be restricted to austere scenarios in which diets would be composed from only 4 to 5 plant species, and all foods would have to be processed from them. this restriction of choices caused the Principal Investigator of NCC 2-100 to re-evaluate further work with lettuce as a CELSS candidate species. It clearly belongs in a generous diet scenario, and could be justified in a more modest one, but could not be in a severe scenario. Thus, research with lettuce was abandoned in favor of some new candidate species with more obvious food value. At that time, the other candidate species being investigated elsewhere included a legume (soybean), a cereal (wheat), and two storage root/tuber calorie crops (sweet potato, potato). The program manager wanted someone to look for another oilseed crop because soybean was the only species with any oil to speak of. Therefore, a species survey was conducted in the greenhouse, screening different cultivars of species such as safflower, peanut, and rapeseed. A number of alternative legumes also were evaluated, including garden pea, garden bean, and cowpea.

An oilseed crop selected for further study was rapid-cycling brassica, a dwarf form of *Brassica napus*. Germplasm was obtained from Dr. Paul Williams of the University of Wisconsin. The small, hard, angular seeds of this dwarf rapeseed did not lend well to direct-seed hydroponics, so a method was devised that employs a solid matrix composed of a perlite: vermiculite mixture and bottom capillary wicking of nutrient solution into the root zone. Dwarf, rapid-cycling brassicas (RCBs) were grown in growth chambers at several different planting densities, different nitrogen concentrations, and at different developmental stages, $\pm$ CO_2 enrichment, and hormone treatments to enhance the onset of senescence to hasten harvest. The oil content of RCB seeds varied inversely with nitrogen concentration of the hydroponic solution. High nitrogen nutrition favored protein accumulation in the seeds at the expense of oil; nitrogen starvation favored oil accumulation at the expense of protein. The RCBs are attractive candidates because they go from seed to flower in less than 3 weeks and from seed to dry, harvestable seed in less than 50 days. Plant height is 15 to 20 cm. This species still has a harvest index less than 30%, but an increase in seed set could increase harvest index significantly. Control of rootzone pH in the solid matrix promises to further enhance yield potential of the RCBs.

A low-fat legume alternative to soybean is the cowpea (*Vigna unguiculata*), which has been screened both in the greenhouse and in the growth chamber. Both high-productivity bush and vining types provided by Dr. B.B. Singh of the International Institute of Tropical Agriculture have been tentatively selected for productivity studies in hydroponic culture in growth chambers. Foliage as well as seeds of cowpeas is edible, and seeds can be eaten as a snapbean as well as a dry bean. Mixed-harvest scenarios of leaf harvest together with seed harvest have been tested and compared with all-vegetative or all-reproductive harvest. Mixed harvest appears to be feasible provided that harvested leaves first are allowed to fully expand and contribute to photosynthetic sinks before leaf stripping takes place. Preliminary studies with CO_2 enrichment indicated substantial enhancement of both vegetative and reproductive yield by cowpea. This species has high complex carbohydrate/low fat/moderate protein contents in leaves as well as seeds. Thus, potential harvest index is very high, and this species is quite promising for further work.

Work with both brassica and cowpea are continuing, along with rice, as part of a larger effort in connection with NASA project NAGW-2329, which is the NASA Specialized Center of Research and Training (NSCORT) in Bioregenerative Life Support at Purdue University.

Conference Proceedings during NCC 2-100:

Bubenheim, D.L., C.A. Mitchell, and S.S. Nielsen. 1990. Utility of cowpea foliage in a crop production system for space, from Proceedings of the First National Symposium NEW CROPS: Research, Development, Economics, Indianapolis, Indiana, October 23-26, Timber Press, Portland, OR.

Mitchell, C.A., S.S. Nielsen, and D.L. Bubenheim. 1990. Environmental modification of yield and food composition of cowpea and leaf lettuce, from Proceedings of the CELSS '89 Workshop, Orlando, Florida, February 5-8, NASA TM 102277.

Mitchell, C.A., S.L. Knight, and T.L. Ford. 1986. Optimization of controlled environments for hydroponic production of leaf lettuce for human life support in CELSS, from Proceedings of the CELSS '85 Workshop, NASA Ames Research Center, Moffett Field, California, July 16-19, NASA TM 88215.

Dissertations Completed during NCC 2-100:

Knight, S.L. 1986. Effects of radiation and CO_2 level on yield, gas exchange, and growth of 'Waldmann's Green' lettuce in Minitron II chambers. Ph.D. Degree from Purdue University, August, 1986.

Refereed Publications in the Open Literature:

Knight, S.L. and C. Mitchell. 1992. Growth responses of leaf lettuce seedlings to benzyl adenine and/or gibberellic acid. HortScience (submitted for publication).

Mitchell, C.A., T. Leakakos, and T.L. Ford. 1991. Modification of yield and chlorophyll content in leaf lettuce by HPS radiation and nitrogen treatments. HortScience 26:1371-1374.

Knight, S. and C. Mitchell. 1988. Growth and yield characteristics of 'Waldmann's Green' leaf lettuce under different photon fluxes from metal halide or incandescent + fluorescent radiation. Scientia Horticulturae 35:51-61.

Knight, S. and C. Mitchell. 1988. Effects of incandescent radiation on photosynthesis, growth rate, and yield of 'Waldmann's Green' leaf lettuce. Scientia Horticulturae 35:37-39.

Knight, S. and C. Mitchell. 1988. Effects of CO_2 and photosynthetic photon flux on yield, gas exchange, and growth rate of 'Waldmann's Green' leaf lettuce. J. Exp. Bot. 39:317-328.

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